OMEGA bio-tek

Innovations in nucleic acid isolation

Product Manual

E.Z.N.A.® HP Tissue DNA Maxi Kit

D5196-00	2 preps
D5196-01	10 preps

April 2013

For research use only.Not intended for diagnostic testing.

E.Z.N.A.® HP Tissue DNA Maxi Kit

Table of Contents

Introduction and Overview.....2
Kit Contents/Storage and Stability.....3
Preparing Reagents.....4
E.Z.N.A.® HP Tissue Maxi Kit Protocol.....5
Troubleshooting Guide.....9

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Introduction and Overview

The E.Z.N.A.® HP Tissue DNA Maxi Kit is designed for efficient recovery of genomic DNA up to 60 kb in size from tissue samples up to 2 grams in size. The specially formulated buffer system ensures the optimal lysis of tissues rich in fat, polysaccharides, and fibers such as brain, adipose, and muscle. This kit can also isolate DNA from molluscs, insects, arthropods, roundworms, flatworms, and other invertebrate tissue samples rich in mucopolysaccharides. The procedure relies on the well established properties of the cationic detergent, cetyltrimethyl ammonium bromide (CTAB), in conjunction with the selective DNA binding of Omega Bio-tek's HiBind® matrix.

Samples are homogenized and lysed in a high salt buffer containing CTAB and digested with proteinase. After addition of chloroform, the homogenate is separate into aqueous and organic phases by centrifugation. The upper, aqueous phase is extracted and BL Buffer is added to provide appropriate binding conditions. The sample is then transferred to the HiBind® DNA Maxi Column, where the genomic DNA binds to the membrane and salt and other contaminants are efficiently washed way. High-quality genomic DNA is eluted with Elution Buffer or water. Purified DNA is suitable for most downstream applications such as endonuclease digestion, thermal cycle amplification, and hybridization techniques.

Each HiBind® DNA Maxi column can bind approximately 2.5 mg genomic DNA. Using greater than 2 g tissue or 5 x 10^8 cells is not recommended.

New in this Edition:

- This manual has been edited for content and redesigned to enhance user readability.
- Proteinase K is now supplied in a liquid form eliminating the resuspension step to prior to use.
- Proteinase K Solution can also be stored at room temperature for 12 months.
- Proteinase Storage Buffer is no longer included in the kit.
- Equilibration Buffer is no longer included with this kit. An optional Column Equilibration Protocol has been added to the protocol for your convenience.
- Equilibration Buffer is replaced with 3M NaOH provided by the user.

Kit Contents

Product	D5196-00	D5196-01
Purifications	2	10
HiBind® DNA Maxi Columns	2	10
50 mL Collection Tubes	2	10
MTL1 Buffer	20 mL	100 mL
BL Buffer	20 mL	100 mL
HB Buffer	28 mL	4 x 28 mL
Proteinase K Solution	750 µL	3.5 mL
RNase A	65 µL	320 µL
DNA Wash Buffer	15 mL	2 x 30 mL
Elution Buffer	10 mL	90 mL
User Manual	✓	✓

Storage and Stability

All E.Z.N.A.® HP Tissue DNA Maxi Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. RNase A must be stored at 2-8°C. Proteinase K Solution can be stored at room temperature for 12 months. For long-term storage (>12 months), store at 2-8°C. Store all other components at room temperature (22-25°C). Check buffers for precipitates before use. Redissolve any precipitates by warming to 37˚C.

- Dilute DNA Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
D5196-00	60 mL
D5196-01	120 mL

E.Z.N.A.® HP Tissue DNA Maxi Kit Protocol

Materials and Equipment to be Supplied by User:

- Centrifuge equipped with swing bucket rotor capable of 5,000 × *g*
- Sterile 50 mL centrifuge tubes
- Water bath capable of 60°C
- Mortar and pestle
- Liquid nitrogen
- 100% ethanol
- Sterile deionized water
- Chloroform
- Isoamyl alcohol
- Optional: RNase A (25 mg/mL)
- Optional: 3M NaOH

Before Starting:

- Prepare DNA Wash Buffer according to the directions in the "Preparing Reagents" section on Page 4.
- Prepare chloroform:isoamyl alcohol (24:1)
- Heat water bath to 60°C

Samples preserved in formalin should be rinsed in xylene and then ethanol before processing. Note that results obtained with formalin-fixed tissues generally depend on the age and size of the specimen. Purified material is usually adequate for PCR amplification, but fresh or frozen samples should be used for Southern analyses.

1. Pulverize 500 mg tissue in liquid nitrogen with mortar and pestle and transfer the powder in a clean 50 mL centrifuge tube. Sample can also be ground and homogenized by beads mill.

   Note: The amount of starting material depends on the sample and can be increased if acceptable results are obtained with the suggested 500 mg tissue. In any event, use no more than 2 g tissue per HiBind® DNA Maxi Column as DNA binding capacity (2.5 mg) may be exceeded. Difficult tissues may require starting with less than 500 mg tissue and doubling all buffer volumes to ensure adequate lysis.

2. Add 9 mL MTL1 Buffer and 300 µL Proteinase K Solution. Vortex to mix thoroughly.

3. Incubate at 60°C for a minimum of 2 hours or until the entire sample is solubilized.

   Note: Actual incubation time varies and depends tissue type. Most samples require no more than 4 hours. Alternatively, an overnight incubation at 55°C will produce adequate results.

4. Add 9 mL chloroform:isoamyl alcohol (24:1). Vortex at maximum speed for 15 seconds.

5. Centrifuge at 4,000 x *g* for 5 minutes at room temperature.

6. Carefully transfer the upper aqueous phase to a clean 50 mL centrifuge tube. Avoid the milky interface containing contaminants and inhibitors. In most cases, ~ 6 mL upper phase can be transferred.

   Note: This step will remove much of the polysaccharides and proteins from solution and improve spin column performance downstream. If little of the upper aqueous phase separates, add 2 mL MTL1 Buffer and vortex to mix. Centrifuge as above and transfer the upper aqueous solution into a new tube.

Optional: Certain tissues such as liver tissue have high levels of RNA which will be co-purified with DNA using this kit. While it will not interfere with PCR, the RNA may be removed at this point.

1. Add 30 µL RNase A (25 mg/mL) per 500 mg tissue.
2. Let sit at room temperature for 5 minutes.
3. Proceed to Step 7 below.

7. Add equal volume BL Buffer. Vortex to mix thoroughly.

   Note: A wispy precipitate may form upon the addition of BL Buffer. This is does not interfere with DNA recovery.

8. Incubate at 70°C for 10 minutes.

9. Add equal volume 100% ethanol. Vortex at maximum speed for 30 seconds.

   Note: If precipitation can be seen at this point, break the precipitation by passing through a needle and syringe.

Optional Protocol for Column Equilibration:

1. Add 3 mL 3M NaOH to the HiBind® DNA Maxi Column.
2. Centrifuge at 4,000 x *g* for 3 minutes.
3. Discard the filtrate and reuse the collection tube.

10. Transfer the sample from Step 9, including any precipitation that may have formed, to a HiBind® DNA Maxi Column.

11. Centrifuge at 4,000 x *g* for 5 minutes at room temperature.

12. Discard the filtrate and reuse the collection tube.

13. Repeat Steps 10-12 until all the remaining sample has been transferred to the HiBind® DNA Maxi Column.

14. Add 10 mL HB Buffer.

15. Centrifuge at 4,000 x *g* for 5 minutes at room temperature.

16. Discard the filtrate and reuse the collection tube.

17. Add 15 mL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with ethanol before use. Please see Page 4 for instructions.

18. Centrifuge at 4,000 x *g* for 5 minutes at room temperature.

19. Discard the filtrate and reuse the collection tube.

20. Repeat Steps 17-19 for a second DNA Wash Buffer wash step.

21. Centrifuge the empty HiBind® DNA Maxi Column at 4,000 x *g* for 10 minutes at room temperature to dry the column.

    Note: This step is critical for removal of trace ethanol that may interfere with downstream applications**.**

22. Transfer the HiBind® DNA Maxi Column to a clean 50 mL centrifuge tube.

23. Add 2-4 mL Elution Buffer (10 mM Tris buffer, pH 8.5) or water heated to 60°C directly onto the center of HiBind® DNA Maxi Column matrix.

24. Let sit for 2-5 minutes at room temperature.

25. Centrifuge at 4,000 x *g* for 5 minutes at room temperature.

26. Repeat Steps 23-25 for a second elution step.

27. Store DNA at -20°C.

    Note: Typically a total of 400 µg DNA with absorbance ratio (A_{260}/A_{280}) of 1.7-1.9 can be obtained from 500 mg animal tissue. Yields vary depending on source and quantity of starting material used.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **1-800-832-8896**.

Problem	Cause	Solution
Clogged Column	Incomplete lysis	Increase incubation time with MTL1 Buffer and Proteinase K Solution. An overnight incubation may be necessary. Centrifuge to remove any insoluble particles.
	Sample too large	Do not use greater than the recommended amount of starting material. For larger samples, divide into multiple tubes.
	Incomplete homogenization	Pulverize starting material as indicated in liquid nitrogen to obtain a fine powder.
Problem	**Cause**	**Solution**
No DNA eluted	Clogged column	See above
	Poor elution	Repeat elution or increase elution volume. Incubation of column at 70°C for 5 minutes with dH_2O or Tris buffer prior to centrifugation may increase yields.
	Poor binding to column	Follow the protocol closely when adjusting the binding conditions. Adjust volumes of BL Buffer and ethanol in proportion.
	Improper washing	DNA Wash Buffer must be diluted with 100% ethanol as specified on Page 4 before use.
Problem	**Cause**	**Solution**
Low A_{260}/A_{280} ratio	Extended centrifugation during elution step	Resin from the column may be present in eluate. Avoid centrifugation at speeds higher than specified. The material can be removed from the eluate by centrifugation. It will not interfere with PCR or restriction digests.
	Poor cell lysis	Increase incubation time with MTL1 Buffer and Proteinase K Solution. An overnight incubation may be necessary.
	Trace protein contaminants remain	Following Step 13, wash the column with a mixture of 300 µL BL Buffer and 300 µL 100% ethanol before proceeding to Step 14.

Troubleshooting Guide

Problem	Cause	Solution
No DNA eluted	Poor cell lysis	Increase incubation time with MTL1 Buffer and Proteinase K Solution. An overnight incubation may be necessary.
	Incomplete homogenization	Pulverize starting material as indicated in liquid nitrogen to obtain a fine powder.
	100% ethanol not added before transferring sample to column	Before transferring the sample to the column, add BL Buffer and 100% ethanol as indicated in Steps 7-9.
	100% ethanol was not added to DNA Wash Buffer	Dilute DNA Wash Buffer with the indicated volume of 100% ethanol before use.

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